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File: USPT

Jul 2, 1996

DOCUMENT-IDENTIFIER: US 5532142 A

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TITLE: Method of isolation and purification of fusion polypeptides

Brief Summary Text (3):

The invention relates generally to the field of molecular biology and to methods of isolating and purifying recombinant proteins. The invention also includes DNA constructs engineered to include DNA encoding a desired polypeptide and a unique plant virus proteinase recognition site. Expression vectors for use in overproducing recombinant plant virus proteinase, particularly tobacco etch virus proteinase, are also part of the invention. The recombinant plant virus proteinase is unaffected by cell protease inhibitors, thereby permitting isolation of significantly improved yields of proteolytically sensitive polypeptides in the presence of added cell protease inhibitors. Methods of overproducing plant viral proteinases are also disclosed.

Drawing Description Text (11):

FIG. 10 shows the purification of histidine-tagged TEV after nickel agarose column purification. Lane 1: molecular weight markers; lane 2: pellet after cell lysis; lane 3: supernatant after cell lysis; lane 4: nickel-agarose column flow-through; lane 5: column flow-through after washing; lanes 6-10: several fractions eluted with 75 mM imidazole, pH 7.0.

Detailed Description Text (33):

Growth conditions and cell lysis for the histidine-tagged TEV proteinases from pGEX-6hispro and pTRC-7hispro were the same as for the GST-tagged proteinase. Absorption to a Nickel NTA-agarose resin was in accordance with the manufacturer's instructions (Quiagen, Chatsworth, Calif.). The His-tagged TEV proteinase was eluted with 75 mM imidazole, pH 7.0. Yields were comparable to the GST-tagged TEV proteinase. SDS-PAGE analysis of a typical purification of histidine-tagged TEV protease is shown in FIG. 10.

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L2: Entry 3 of 19

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May 6, 2003

DOCUMENT-IDENTIFIER: US 6559176 B1

TITLE: Compounds and methods for regulating bacterial growth and pathogenesis

Detailed Description Text (43):

"Antifungal agents" means systemic antifungal agents such as amphotericin B, flucytosine, imidazoles and triazoles, ketoconazole, miconazole, itraconazole, fluconazole and the like. This category also includes griseofulvin. We also mean topical antifungal agents such as clotrimazole, econazole, miconazole, terconazole, butoconazole, ciclopirox olamine, haloprogin, tolnaftate, naftifine, terbinafine as well as nystatin, amphotericin B, undecylenic acid, benzoic acid, salicyclic acid, propionic acid, caprylic acid and potassium iodide.

Detailed Description Text (93):

The protein produced by luxS gene expression in a recombinant procaryotic or eucyrotic system may be purified according to methods known in the art. In a preferred embodiment, a commercially available expression/secretion system can be used, whereby the recombinant protein is expressed and thereafter secreted from the host cell, to be easily purified from the surrounding medium. If expression/secretion vectors are not used, an alternative approach involves purifying the recombinant protein by affinity separation, such as by immunological interaction with antibodies that bind specifically to the recombinant protein. Such methods are commonly used by skilled practitioners.

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L1: Entry 2 of 118

File: USPT

May 27, 2003

DOCUMENT-IDENTIFIER: US 6569631 B1

TITLE: Microplate thermal shift assay for ligand development using
5-(4"dimethylaminophenyl)-2-(4'-phenyl)oxazole derivative fluorescent dyesDetailed Description Text (95):

Techniques for isolating inclusion bodies, purifying recombinant protein from inclusion bodies, and techniques for refolding or renaturing protein are well known to those skilled in the art. For example, see Sambrook, J. et al., Molecular Cloning: a Laboratory Manual, pp. 17.37-17.41, Cold Spring Harbor Laboratory Press (1989); Rudolph, R. et al., FASEB J. 10:49-56 (1995).

WEST**End of Result Set**

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L3: Entry 1 of 1

File: USPT

Dec 29, 1998

DOCUMENT-IDENTIFIER: US 5854081 A

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TITLE: Stable expression of human A.sub.2B adenosine receptors, and assays employing the same

Detailed Description Text (20):

It has been found that recombinant A.sub.1 adenosine receptors engineered to contain hexahistidine at either the amino or carboxyl terminus could be purified to 200- to 300-fold, not enough to effect purification to homogeneity. A second means of purifying recombinant proteins is to engineer in an antibody epitope. Numerous epitopes have been used. The FLAG system utilizes immobilized anti-FLAG antibodies to purify recombinant proteins containing the eight amino acid FLAG epitope (Asp-Try-Lys-Asp-Asp-Asp-Lys). The FLAG peptide can be cut after its C-terminal amino acid by enterokinase, and so removed from the amino terminus of purified proteins. The preparation of vectors designed to express receptors extended on the amino terminus with hexahistidine and the FLAG epitope is described herein. This extension has been added to human A.sub.1, A.sub.2A, A.sub.2b, A.sub.3 adenosine receptors and found not to alter ligand binding properties and to permit the purification of the H/F-A.sub.1 and H/F-A.sub.2A receptors in high yield and to high purity. The degree of purification achieved by the one of both FLAG and hexahistidine markers, has not been achieved using prior art single tags. The receptors have been purified to homogeneity, based on silver staining of SDS polyacrylaminate gels.

Detailed Description Text (44):

Elution fractions (2 mL) from an anti-FLAG column were loaded by gravity onto 1 mL Ni-NTA-agarose columns prewashed with buffer CD plus 1 mM imidazole. Columns were washed four times with 1.5 ml of wash buffer and eluted with 5.times.1 mL of the same buffer supplemented with 200 mM imidazole, pH 7.4.

Detailed Description Text (46):

Elution fractions (500 .mu.L) from anti-FLAG columns containing H/F-A1 were added to microfuge tubes containing 250 .mu.L Ni-NTA-agarose prewashed three times with buffer CD plus 1 mM imidazole. After incubating on a rocker for 40 minutes at 4.degree., the tubes were centrifuged at 5000 g for 10 minutes, and the supernatant was removed. The resin was washed twice with 800 .mu.L of buffer CD plus 1 mM imidazole and eluted two times with 200 .mu.L of buffer CD plus 200 mM imidazole.

Detailed Description Text (63):

To test the efficiency of anti-FLAG antibody columns and Ni-NTA columns to retain H/F-modified adenosine receptors, human H/F-A.sub.1 receptor was used as a prototype. Small batches of membranes made from CHO-K.sub.1 cells expressing native and H/F-A.sub.1 receptors were photoaffinity labeled with the A.sub.1 selective antagonist .sup.125 I-Azido-BW-A844U. After digitonin treatment, the soluble fraction was loaded twice on an anti-FLAG affinity column. Following extensive washing, the receptor was eluted in four 1-mL fractions, each containing 200 .mu.g of the fraction and by autoradiography following SDS-PAGE. Digitonin was found to solubilize 50-60 percent of specific [.sup.3 H]CPX binding sites. No photoaffinity labeled native-A.sub.1 receptors adhered to anti-FLAG columns, due presumably to the absence of the FLAG epitope. 80 percent of photoaffinity-labeled H/F-A.sub.1 receptors adhered to the anti-FLAG column. Of the retained receptors, 65 percent were eluted with the FLAG peptide in fractions 1-2 and >90 percent in fractions 1-4. A broad major band was seen after gel electrophoresis of the load and the elution fractions with an apparent molecular mass of 38-43 kDa. After deglycosylation with N-glycosidase F, the labeled protein was quantitatively shifted to a molecular mass of 33-34 Kda. These findings are reasonably

consistent with the deduced molecular mass of the H/F-A.sub.1 receptor, 38.8 Kda, of which 2.26 kDa is derived from the H/F extension. The results also are in agreement with previous photoaffinity labeling experiments. Patel et al, supra. Application of crude digitonin-solubilized receptors to Ni-NTA columns resulted in poor retention (<25 percent) of the photoaffinity-labeled receptors. This is attributed to relatively low affinity of the Ni-NTA for the receptor, resulting in gradual elution of receptors during the loading of large volumes (>4 column volumes) of receptors to the Ni-NTA column. Ni-NTA was much more efficient when used as a second affinity column step with concentrated receptors.

Detailed Description Text (65):


Ni-NTA chromatography of receptors previously purified using anti-FLAG columns was useful to achieve somewhat higher purification. However, when loaded onto Ni-NTA columns, it was necessary to keep load and wash volumes small (<4 column volumes) to prevent leaching of H/F receptor off the Ni-NTA resin. An efficient microfuge tube procedure was developed as follows: Receptors eluted from an anti-FLAG column (1 mL) were mixed with 0.5 mL Ni-NTA resin, washed two times with 0.8 mL wash buffer, and eluted by twice adding 200 .mu.L buffer plus 200 mM imidazole (see Materials and Methods). By this procedure, the recovery of photolabeled H/F-A.sub.1 adenosine receptor was >80 percent.

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


















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

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